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Review Article

Gene-Environmental Interactions: Lessons from Porphyria

Shigeru SASSA

Associate Professor Emeritus and Head of Laboratory, Laboratory of Biochemical Hematology, The Rockefeller University, New York, U.S.A.

Abstract

The porphyrias are uncommon, complex, and fascinating metabolic conditions, caused by deficiencies in the activities of the enzymes of the heme biosynthetic pathway. Two cardinal symptoms of the porphyrias are cutaneous photosensitivity and neurologic disturbances. Molecular analysis of gene defects has shown that there are multiple and heterogeneous mutations in each porphyria. Patients with symptomatic porphyria can suffer greatly, and, in rare cases, may die. While congenital porphyrias are inherited, other forms of porphyria occur as acquired diseases. In addition, not all gene carriers of inherited porphyrias develop clinical disease and there is a significant interplay between the gene defect and acquired or environmental factors. The variable response of porphyrias to acquired factors may likely reflect genetic polymorphisms in drug metabolism. The lessons from acute hepatic porphyria, such as acute intermittent porphyria, are very useful in clarifying the complex nature of the clinical expression of metabolic disorders.

Key words: porphyria, acute intermittent porphyria, cytochrome P450, CYP, polymorphism, gene-environmental interaction

Introduction

The concept of the gene-environmental interaction was probably first couched by Archibald Garrod. Garrod was considered the father of biochemical genetics since he recognized that genetics, biochemistry and medicine are interacting disciplines, and coined the term, *Inborn Errors of Metabolisms*, for such interactions. Garrod's devotion to the chemical abnormalities found in the urine of rare inherited disorders, such as alcaptonuria, led him to form the basis of a new concept and the Croonian Lectures on *Inborn Errors of Metabolism*, in 1908 (1). In this concept, he clearly saw the gene-environmental interaction plays an important role in biological science and medical practice. Later, he added porphyrias to the list of inborn errors of metabolism.

Here, I review the current knowledge of a human porphyria, acute intermittent porphyria (AIP), and lay out how much one could learn about gene-environmental interaction from the analysis of this rare 'monogenic' disorder.

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Reprint requests to: Shigeru SASSA
Yamanouchi Pharmaceutical Co., Ltd., 17-1, Hasune 3-Chome, Itabashi-Ku, Tokyo 174-8612, Japan
TEL: +81(3)5916-5309, FAX: +81(3)5916-5607
E-mail: sassas@yamanouchi.co.jp
Abbreviations used: AIP, Acute intermittent porphyria; ALA, δ-Aminolevulinic acid; ALAD, δ-Aminolevulinate dehydratase; ALAS, δ-Aminolevulinate synthase; CYP, Cytochrome P450; EM, Extensive metabolizer; FAA, Fumarylacetoacetate; HO-1, Heme oxygenase-1; HT1, Hereditary tyrosinemia type 1; PBG, Porphobilinogen; PBGD, Porphobilinogen deaminase; PM, Poor metabolizer; SA, Succinylacetone.

Acute intermittent porphyria

The porphyrias are uncommon, complex, and fascinating metabolic conditions, caused by deficiencies in the activities of enzymes of the heme biosynthetic pathway (2). Patients with symptomatic porphyria can suffer greatly, and, in rare cases, may die. However, not all gene carriers of inherited porphyrias develop clinical disease and only a small fraction of gene carriers eventually develop the clinical disease. Thus, there is a significant interplay between the gene defect and environmental factors. This situation is best exemplified in the case of AIP.

Historical aspects

Perhaps the oldest description of porphyria, which was most likely AIP, can be found in the medical record by Hippocrates in 400 B.C., who described a woman from *Thasos* having a repeated history of "dark urine" with highly variable forms of spasms and psychological symptoms. Her symptoms and clinical course were compatible with acute attacks of hepatic porphyria such as AIP (3). In 1888, Sulfonal (Fig. 1) was introduced as a new hypnotic into the market (4). Soon after, many patients died from acute attacks, presumably of AIP, after taking Sulfonal. For example, 7 women died in 1889/1890, after taking Sulfonal, at a private neuropsychiatric hospital in Inzerdorf, Germany (4).

AIP is an autosomal dominant disorder resulting from a partial deficiency of porphobilinogen deaminase (PBGD) activity (5). Typically the deficient enzyme activity is about 50% of normal, and is found in all tissues in the great majority of patients (95% or greater). The cardinal pathobiologic defect of the disease is a neurologic dysfunction that may affect the peripheral, autonomic

Fig. 1 The label of Sulfonal marketed by Bayer in 1888, as a sleep inducer. The drug was soon removed from the market because of many acute attacks of AIP (4).

and central nervous systems. The majority (greater than 90%) of individuals with this inherited enzyme deficiency, however, remain clinically normal throughout life. The clinical expression of the disease is usually linked to environmental or acquired factors, e.g., nutritional status, drugs, sex steroids and other chemicals of endogenous or exogenous origin, suggesting that there is a significant environmental effect on the clinical expression of the primary gene defect, namely the inherited PBGD deficiency.

AIP is the severest form of the acute hepatic porphyrias and probably the most common of the genetic porphyrias. The prevalence of AIP in USA was reported to be 5–10 per 100,000 (5). The highest incidence of AIP occurs in Lapland, followed by Scandinavia and the United Kingdom, although it has been reported in many population groups, including Japanese (6). The disorder is expressed clinically almost invariably after puberty and more often in women than in men.

PBGD is the third enzyme in the heme biosynthetic pathway, and catalyzes the condensation of four molecules of PBG to yield a linear tetrapyrrole, hydroxymethylbilane (Fig. 2). In the presence of the subsequent enzyme, uroporphyrinogen III cosynthase, hydroxymethylbilane is converted to uroporphyrinogen III with inversion of the D ring pyrrole. There are two isozymes of PBGD: erythroid-specific and nonspecific (7). The two isoforms of PBGD are produced by distinct messenger RNAs (mRNAs), which are transcribed from a single gene by alternate transcription and splicing of its mRNA. The human erythroid-specific PBGD consists of 344 amino acid residues; nonspecific PBGD contains 17 additional amino acid residues at its N-terminus, while the remainder are identical to the erythroid enzyme (Fig. 3) (8).

Clinical and Biochemical Findings

Abdominal pain, which may be generalized or localized, is the most common symptom and is often the initial symptom of an acute attack. Other gastroenterologic features may include nausea, vomiting, constipation or diarrhea, abdominal distention and ileus. Urinary retention, incontinence and dysuria may frequently be observed. Tachycardia and hypertension and, less often, fever, sweating, restlessness and tremor are also observed. Peripheral neuropathy and muscle weakness are also common features of AIP. Acute attacks of AIP may be accompanied by seizures, especially in patients with hyponatremia due to vomiting, inappropriate fluid therapy, or the syndrome of inappropriate antidiuretic hormone release. No cutaneous manifestations are associated with this enzyme deficiency. Reflecting partial PBGD deficiency, patients with clinically expressed AIP excrete increased amounts of ALA and PBG in the urine during attacks and sometimes between attacks. In severe cases, the urine develops a portwine color from a high content of porphobilin, an autooxidation product of PBG.

Molecular Biology

More than 170 different point mutations of the human *PBGD* gene have been described in AIP. Most mutations were found separately in each different family, thus they are "private", while only few represent a founder mutation which affects more than one pedigree. Patients with AIP can be classified into three subsets. Patients with type I mutations are characterized by cross-reactive immunologic material (CRIM) negative PBGD mutations, and they exhibit both reduced enzyme activity and reduced PBGD protein content (about 50% of normal). Mutations found in type I AIP are mostly singlebase substitutions or deletions that lead to a single amino acid change or to truncated proteins, which result in the loss of expression of the enzyme protein (Fig. 4). Patients with type II mutations are observed in fewer than 5% of all AIP patients and are characterized by decreased PBGD activity in nonerythroid cells, such as liver (about 50% of normal), but these patients have normal erythroid PBGD activity. The mutations found in type II AIP are singlebase substitutions that occur in the exon/intron boundary of exon 1, resulting in a splicing defect that affects the nonspecific form of PBGD, but not the erythroidspecific PBGD, because the transcription of the gene starts downstream from the site of mutation (9) (Fig. 4). Patients with type III mutations are characterized by CRIM positive mutations, that is, decreased enzyme activity (about 50% of normal) with the presence of structurally abnormal enzyme protein (10). Mutations characterizing type III AIP, mostly occurring in exons 10 and 12, are observed in the region that is essential for catalytic activity.

Factors that influence the clinical expression of the primary PBGD deficiency

It is clear that an inherited deficiency of PBGD is not in itself sufficient to cause the clinical expression of AIP. The great majority—perhaps greater than 90%—of individuals who inherit a deficiency of PBGD never develop porphyric symptoms. The nature of some of the major factors that are essential for the clinical expression of AIP is evident from the clinical features. Namely, clinical features in individual cases often suggest that when the clinical expression does develop multiple additional factors are contributing in an additive fashion (Fig. 5). Not uncommonly, the immediate precipitating factor cannot be identified. It is possible that individual susceptibility to the clinical expression among those who inherit PBGD deficiency may be determined by other and presently unknown genetic factors, or more likely by genetic polymorphisms in the responses of the individuals to such factors. The major precipitating factors of AIP can be classified as follows. Rarely, AIP may also occur as a result of other inherited enzymatic deficiency.

(1) Endocrine factors

There is considerable evidence that endocrine factors such as steroid hormones are important precipitating factors in AIP. The reasons for this suggestion are as follows: (1) The disease is rarely symptomatic before puberty. Likewise, increased excretion of porphyrin precursors is almost never observed in children with

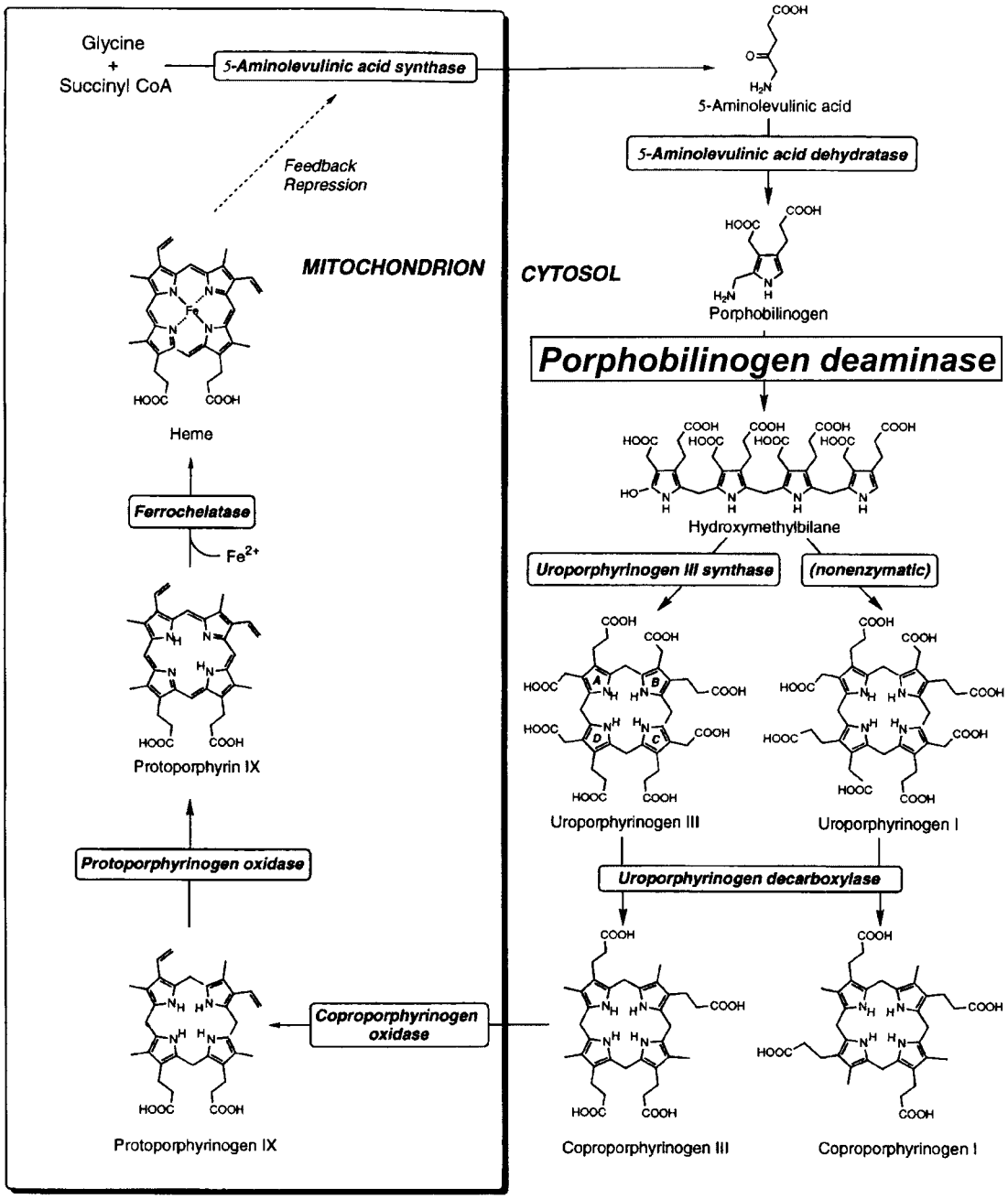

Fig. 2 Enzymes (*italics*) and intermediates (plain letters) in the heme biosynthetic pathway.
Pyrrole ring designation is shown in the structure of uroporphyrinogen III. In uroporphyrinogen III, the substituent groups in the D ring are reversed because the ring orientation has been flipped during the formation of the type III isomer (5). Porphobilinogen deaminase (PBGD) catalyzes condensation of 4 molecules of PBG to form hydroxymethylbilane. Its deficiency results in the accumulation of ALA and PBG, and clinically in AIP.

PBGD deficiency. (2) Symptoms are more common in women with this enzyme deficiency than in men suggesting that adult levels of female hormones are particularly important. (3) Some women develop attacks almost monthly in the premenstrual period. These are probably due to endogenous progesterone, and can be prevented by the administration of gonadotropin-releasing hormone (GnRH) analogs (11). (4) AIP is sometimes exacerbated by exogenous steroids, including oral contraceptive preparations. (5) Pregnancy may exacerbate, but only in a minority of patients. (6) Attacks appear to be less common in women after menopause, although they can occur. (7) More subtle abnormalities in steroid hormone metabolism, such as a deficiency of hepatic steroid 5α-reductase activity, in some patients with AIP can predispose to the excessive production of steroid hormone metabolites that are inducers of δ-aminolevulinate synthase in the liver (12).

Pregnancy is usually well tolerated in AIP. For example, Kauppinen et al. (13) reviewed the clinical features of 76 women with AIP or variegate porphyria with a total of 176 deliveries. Symptoms of porphyria did not occur in 162 (92%) of these pregnancies. The generally favorable course during pregnancy is rather surprising, given the considerably increased circulating levels of progesterone, which is a known inducer of hepatic heme synthesis (14). This rule is, however, not generally applicable since some women with AIP do have attacks during pregnancy, suggesting that there is considerable genetic polymorphism in the response to hormonal changes during pregnancy, including progesterone,

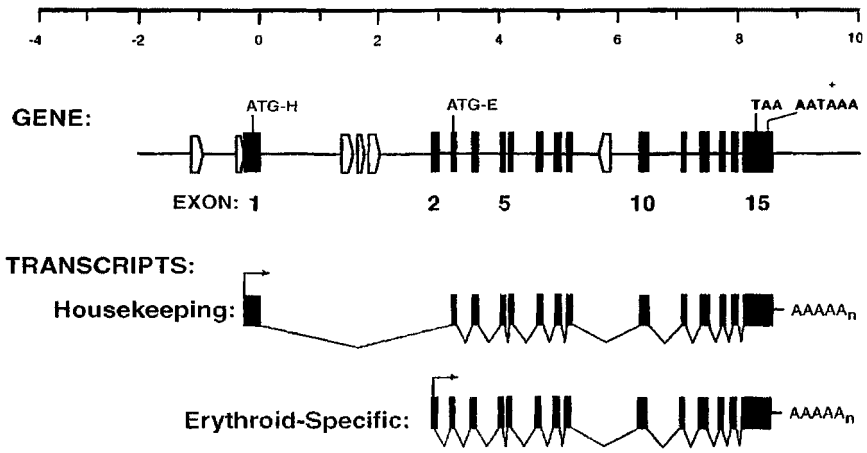

Fig. 3 Organization of the *PBGD* gene and alternate splicing of the housekeeping and the erythroid-specific transcripts (5).
The 15 exons are represented as solid rectangles, and the positions and orientations of *Alu* repeat element are indicated by the pentagonal boxes. The promoter region for the housekeeping transcript is 5' to exon 1, and the promoter for the erythroid-specific transcript is immediately 5' to exon 2. Translation initiation methionines for the housekeeping and the erythroid-specific enzymes are indicated (ATG-H and ATG-E, respectively).

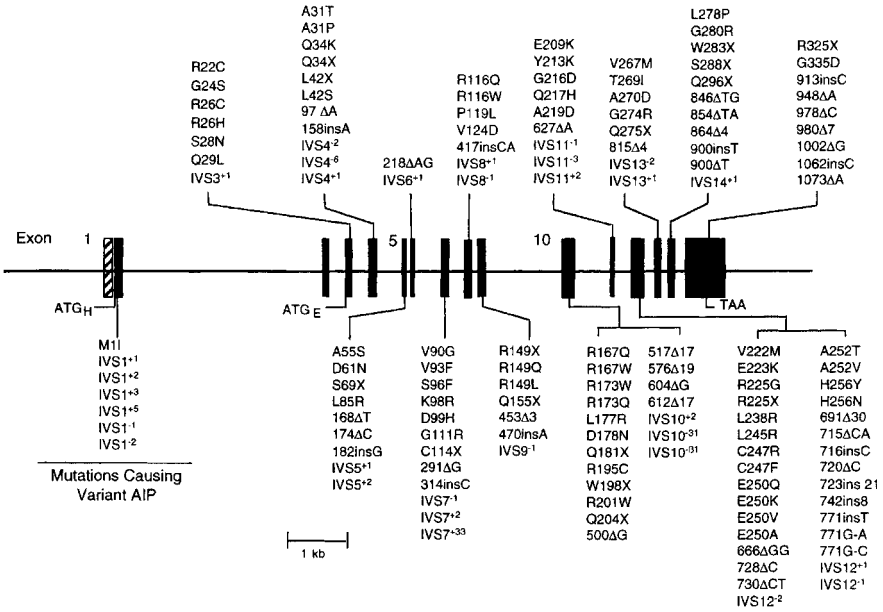

Fig. 4 The human *PBGD* gene, with localizations of some of the mutations found in AIP (5).

among different patients.

(2) Drugs

Drugs are among the most important factors that precipitate acute attacks of AIP. Barbiturates and sulfonamide antibiotics have been the drugs most commonly implicated in causing acute attacks of AIP. Barbiturates are now seldom used as sedatives in medical practice, while sulfonamides and other drugs remain important as precipitating agents of AIP.

A significant number of commonly used drugs are widely agreed to be either *Safe* or *Unsafe*, and are so listed in Table 1. On the other hand, knowledge about the safety of many drugs in AIP is so limited that it is not possible to classify all drugs and foreign chemicals as definitely *safe* or *unsafe*. Thus, some of these are listed as "*Potentially Unsafe*" or "*Probably Safe*" in Table 1. Clinical observations of the effects of drugs in patients with AIP are usually sporadic, incompletely reported and necessarily uncontrolled. Information is also likely to be lacking for recently introduced drugs, so they should be avoided if older drugs are reasonable alternatives for treating concurrent conditions.

Knowing the effects of a drug on hepatic heme metabolism in humans or laboratory animals is often used in predicting whether it is safe or unsafe, however, such information should be interpreted with caution because of possible species specificity. Most drugs that exacerbate AIP have the capacity to induce ALAS1 in the liver. ALAS1 induction in the liver is closely associated with induction of cytochrome P450 enzymes, a process that increases the demand for heme synthesis in the liver (14). If one of the enzymes in the heme biosynthetic pathway is deficient, ALAS1 induction occurs with lower dosages of inducing drugs (15). This situation is particularly dominant in AIP, since hepatic PBGD activity is very close to the rate limiting level in heme synthesis (16). In the liver the response to an increased demand for heme includes induction of ALAS1, which in turn can lead to accumulation of pathway intermediates. Therefore, it is reasonable to consider that any drug that induces hepatic cytochrome P450 enzymes is potentially harmful in AIP, which would accompany induction of hepatic ALAS1 and overproduction of ALA and PBG. Al-

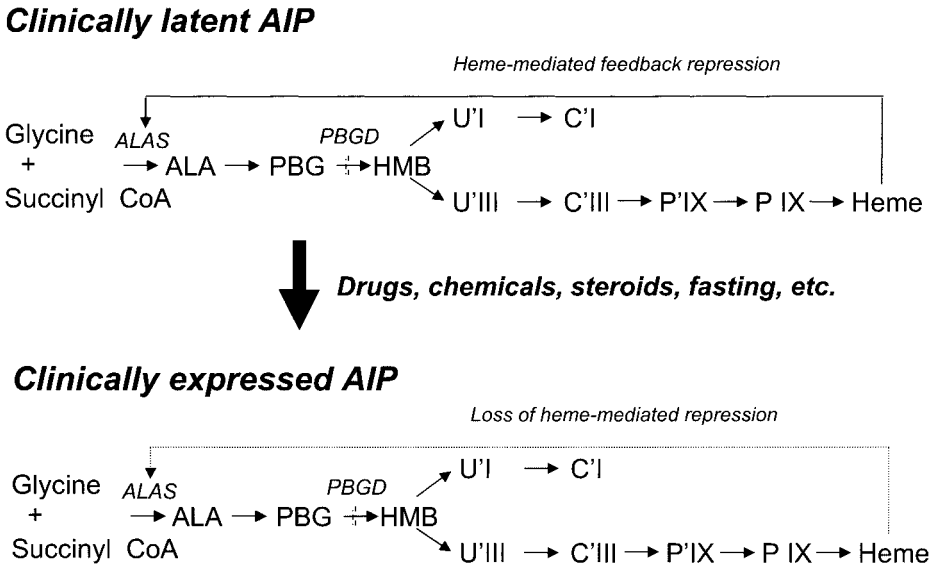

Fig. 5 The enzymatic block in AIP. Loss of heme-mediated repression of hepatic ALAS1 occurs when the disease is clinically manifested by precipitating factors such as drugs, steroids, and dietary restrictions. In the presence of PBGD deficiency, factors that stimulate ALAS1 synthesis, or heme destruction result in decreased availability of heme for the regulatory heme pool in hepatocytes (39).
ALA=δ-aminolevulinic acid; ALAD=δ-aminolevulinate dehydratase; ALAS1=δ-aminolevulinate synthase 1 (housekeeping form); HMB=hydroxymethylbilane; PBG=porphobilinogen; PBGD=porphobilinogen deaminase

though drugs that induce cytochrome P450 enzymes and ALAS1 in the human liver generally also induce ALAS1 in laboratory animals and liver cells in culture, there may be important species and dosage differences that complicate extrapolation of animal data, or finding in tissue culture, to human AIP. In contrast to drugs that induce hepatic ALAS1, drugs that interact with P450 enzymes as substrates or inhibitors are not necessarily harmful in AIP.

Ethanol intake has sometimes been associated clinically with attacks. Ethanol and other alcohols found in beverages are inducers of ALAS1 and at least some cytochrome P450 enzymes (17).

Some drugs can also increase heme synthesis by promoting the destruction of cytochrome P450 enzymes, at least in experimental systems (18). Mechanism-based destruction of cytochrome P450 by some drugs and chemicals can also lead to formation of N-alkylated protoporphyrins, e.g., N-methyl protoporphyrin, are potent inhibitors of ferrochelatase (19). Griseofulvin, for example, is known to be harmful in AIP, perhaps at least in part due to formation of N-methyl protoporphyrin. Inhibition of ferrochelatase by N-alkylated protoporphyrin and destruction of cytochrome P450 can further limit heme synthesis. Some synthetic steroids, when given in high doses to laboratory animals, can also cause mechanism-based destruction of cytochrome P450 enzymes (20).

Anesthetic agents have been studied in animal models and the clinical experience in patients with acute porphyrias were reviewed (21, 22). It is extremely important to avoid induction of anesthesia with a barbiturate, the risk for which is great in patients in whom the diagnosis of AIP has not been recognized prior to surgery. Halothane was recommended as an inhalation agent and propofol or midazolam appear suitable as intravenous induction agents for use in AIP (22). In contrast, even major surgery can be carried out safely in patients known to have AIP when appropriate anesthetic drugs are used (23). Antineoplastic drugs have been generally administered safely to patients with AIP and advanced cancer (24). There is disagreement, however, regarding the safety of several antibiotics such as chloramphenicol, cephalosporins, erythromycin and vancomycin (25).

Even when a harmful drug induces an attack of AIP, other predisposing factors such as endogenous hormones, nutritional factors and smoking have additive effects in a given patient. An important clinical feature of AIP is that multiple inducing factors are almost always involved. Such clinical observations include the following. (i) Harmful drugs and other precipitating factors are less likely to cause attacks in patients with no recent symptoms of AIP than in those with recent and frequent symptoms (13). In a large retrospective study of the risk from anesthetic use in patients with AIP in Finland, barbiturates or other inducing drugs were relatively frequently detrimental in patients who already had porphyric symptoms but seldom exacerbated latent disease (21). (ii) Some PBGD-deficient heterozygotes who require long-term anticonvulsants for epilepsy do not develop attacks of porphyria. (iii) Drugs are only rarely reported to cause acute symptoms in children with PBGD deficiency.

Although clinical experience suggests that individuals with latent AIP are less likely to develop attacks than patients with recently active diseases, it is nonetheless recommended that exposure to harmful drugs be avoided in all PBGD-deficient heterozygotes, including children.

(3) Nutritional factors

Diet and nutritional status are underrecognized as contributors to exacerbations of acute porphyrias, in part because obtaining accurate dietary histories is often difficult. There have also been only few studies of nutrition in patients with these conditions. In metabolic ward studies, reductions in caloric and carbohydrate intake have increased urinary ALA and PBG and precipitated symptoms (26, 27). Reduced energy intake, usually instituted in an effort to lose weight, commonly contributes to attacks of AIP. Therefore, even brief periods of starvation during weight reduction, during postoperative periods, or with intercurrent illnesses should be avoided (27).

Glucose and other forms of carbohydrate are effective in treating acute attacks of AIP. The exact nature of the carbohydrate effect is yet unclear. In animals, starvation enhances, whereas

Table 1 Safe and unsafe drugs in AIP* (1)

Unsafe	Potentially Unsafe	Probably Safe	Safe
ACE inhibitors	Alfadolone acetate	Adrenaline	Acetaminophen
Antipyrine	Alfaxolone	Amitriptyline	Acetazolamide
Aminopyrine	Alkylating agents (chlorambucil and melphalan may be somewhat safer)	Azathioprine	Allopurinol
Aminoglutethimide		Chloramphenicol	Amiloride
Barbiturates (all)	Altretamine (hexamethylmelamine)	Cisapride	Aspirin
N-Butylscopolammonium bromide		Colchicine	Atropine
Calcium channel blockers	Benzodiazepines	Cyclosporin	Bethanidine
Carbamazepine	Busulfan	Cytarabine	Bromides
Chlorpropamide	Captopril	Dicumarol	Bumetanide
Danazol	Cephalosporins	Chloroquine	Chloral hydrate
Dapsone	Chlorambucil (see alkylating agents)	Digoxin	Cimetidine
Diclofenac	Chlordiazepoxide	Daunorubicin	Corticosteroids
Enalapril	Clonidine	Doxazosin	Coumarins
Diphenylhydantoin	Cyclophosphamide	Estrogens (natural/endogenous)	Fluoxetine
Ethosuximide	Diazepam	Ibuprofen	Gabapentin
Ergot preparations	Diltiazem	Indomethacin	Gentamicin
Ethchlorvynol	Colistin	Labetalol	Guanethidine
Ethinamate	Dacarbazine	Lithium	Insulin
Felbamate	Diphenhydramine	Losartan	Narcotic analgesics
Glutethimide	EDTA	Methenamine	Ofloxacin
Griseofulvin	Etomidate	Methylphenidate	Penicillin and derivatives
Ketoconazole	Estrogens (synthetic)	Naproxen	Phenothiazines
Lamotrigine	Erythromycin	Neostigmine	Propranolol
Mephenytoin	5-Fluorouracil	Nortriptyline	Streptomycin
Metoclopramide	Gold compounds	Nitrous oxide	Succinylcholine
Meprobamate	Fluroxene	Penicillamine	Tetracycline
Methyprylon	Heavy metals	Procaine	
Nefazadone	Hydralazine	Propanidid	
Nifedipine	Hyoscine	Propofol	
Novobiocin	Ifosfamide	Propoxyphene	
Phenylbutazone	Iron chelators	Rauwolfia alkaloids	
Primidone	Ketamine	6-Thioguanine	
Pargyline	Lisinopril	Thiouracils	
Progesterone (progestins)	Mefenamic acid	Thyroxine	
Rifampin	Melphalan	Tricyclic antidepressants	
Succinimides	Mifepristone (RU-486)	Tubocurarine	
Sulfasalazine	Methyldopa	Vigabatrin	
Sulfonamide antibiotics	Metyrapone	Vitamin B	
Sulfonmethane (Sulfonal)	Nalidixic acid	Vitamin C	
Sulfonethylmethane (Trional)	Nikethamide		
Sulfonylureas	Nitrazepam		
Trimethadione	Nitrofurantoin		
Valproic acid	*o,p,*'-DDD		
Tranylcypromine	Pentazocine		
	Phenoxybenzamine		
	Procarbazine		
	Pyrazinamide		
	Spironolactone		
	Theophylline		
	Tiagabine		
	Tramadol		
	Tricyclic antidepressants		
	Troglitazone		

* Drugs are listed in 4 categories, depending upon the weight of evidence as to their safety. There is considerable evidence for classification of drugs in the Safe and Unsafe categories, but much less evidence, or conflicting evidence, for drugs in the other 2 categories.

glucose or protein can repress, the inducing effect of chemicals on ALAS1 and on PBG excretion (28). Increased dietary carbohydrates can reduce cytochrome P450 enzymes in normal animals and humans (29). Therefore, it is possible that the demand of hepatic heme synthesis is decreased during carbohydrate feeding. If so, the carbohydrate effect on ALAS1, and therefore the benefi-

cial carbohydrate effect in AIP, may be secondary at least in part to an effect on the synthesis of cytochrome P450 enzymes. Starvation in animals induces HO-I, which can lead to depletion of regulatory hepatic heme pools and contribute to ALAS1 induction.

(4) Smoking

Smokers are exposed to chemicals such as polycyclic aromatic hydrocarbons that induce hepatic cytochrome P450 enzymes and heme synthesis (30). It is well known that drug metabolism by cytochrome P450 enzymes is increased in smokers, reflecting increased amounts in these liver hemoproteins. An association between cigarette smoking and repeated attacks of AIP was found in a survey of 144 patients with AIP in Britain (31). Therefore, smoking cessation may have particular health benefits in patients with AIP.

(5) Infections, surgery and stress

Attacks of porphyria may develop during intercurrent infections and other illnesses and after major surgery. The mechanisms involved in situations of increased metabolic stress are not known, but impaired nutrition and the increased production of steroid hormones that induce ALAS1 may play a role (32). Patients report that psychological stress can contribute exacerbations of porphyria, but underlying mechanisms are not established. It should be also noted that oxidative tissue injuries, such as infections, surgery and oxidative stress, induce HO-1, which may then contribute to the reduction in the regulatory free heme concentration in the liver, and ultimately result in the induction of an acute attack of AIP.

(6) Effect of other gene defects

Peculiarly, AIP is known to occur in association with another inherited disorder, hereditary tyrosinemia type I (hepatorenal tyrosinemia, or HT1). HT1 is an inborn error of tyrosine metabolism with the highest incidence of progression to hepatocellular carcinoma. This is most likely to be due to profound mutagenic effects and influences on the cell cycle by accumulated abnormal metabolites. For example, fumarylacetoacetate (FAA), the mutagenic metabolite accumulating in HT1, induces spindle disturbances and segregational defects in both rodent and human cells. FAA-treated cells developed micronuclei, which were predominantly CREST-positive, suggesting chromosomal instability. A sustained activation of the extracellular signal-regulated protein kinase (ERK) was also observed. The tumorigenic-related phenomenon of FAA was suggested to reflect the biochemical/cellular effects of FAA as a thiol-reacting and organelle/mitotic spindle-disturbing agent. In addition, many patients with HT1 develop clinical symptoms characteristic of AIP. These patients also excrete elevated concentrations of ALA, but not PBG, into urine. Marked ALA excretion is identical to that found in patients with AIP in acute attacks. This phenomenon was suggested to be due to the potent inhibition of δ-aminolevulinate dehydratase (ALAD) activity by succinylacetone (SA), a metabolite downstream from FAA in tyrosine metabolism. SA is 4,6-dioxoheptanoic acid, which is a structural analog of ALA, and is the most potent known inhibitor of ALAD, with a Ki of 0.3 μM (33). ALAD activity was markedly inhibited in the liver (34) and erythrocytes (34) in patients with HT1, and was restored to normal in erythrocytes in a patient following liver transplantation (34). ALA, but not PBG, is excreted in high concentrations in children with HT1 (35). These findings strongly suggest that SA is responsible for clinical development of AIP-like symptoms in HT1.

Genetic polymorphisms and drug response

The reasons that some patients with AIP may fare well during pregnancy while others experienced acute attacks (13), or that a drug, such as amlodipine, is safe in one patient (36) while it is not in another (37), probably reflect genetic polymorphisms in drug metabolism. Recently, a large amount of information on genetic polymorphisms in drug metabolisms has been uncovered. Genetic polymorphism can occur in every phase of drug metabolism which is generally classified into three phases and the majority occurs in the liver (Fig. 6). Phase I is the essential part of drug metabolism, which is typically oxidation of the drug substrate to more hydrophilic metabolites, and in many cases, is catalyzed by cytochrome P450 (CYP), the major hemoprotein in the liver. Phase II is conjugation of the metabolite by various conjugating enzymes which add glucuronide, sulfate, etc., to the metabolite which result in a

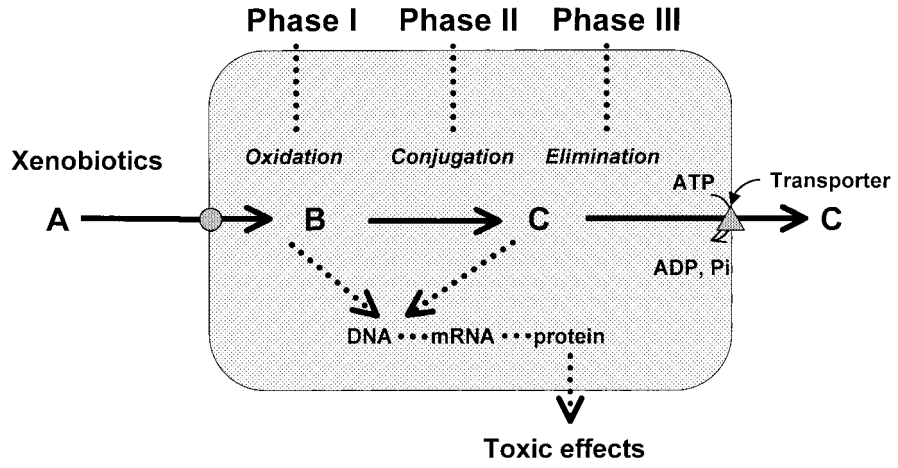

Fig. 6 Three phases in drug metabolism
Phase I is typically oxidation of the drug substrate to more hydrophilic compounds, and largely catalyzed by microsomal cytochrome P450. Phase II is conjugation of the metabolite by various conjugating enzymes which include glucuronidation, sulfation, methylation, acetylation, etc. Phase III is elimination of the conjugated metabolites from the cell which involves a transporter protein on the cell membrane. If a metabolite is toxic, drug metabolism contributes to the toxic effects of the drug.
Both circle and triangle symbols represent transporters.

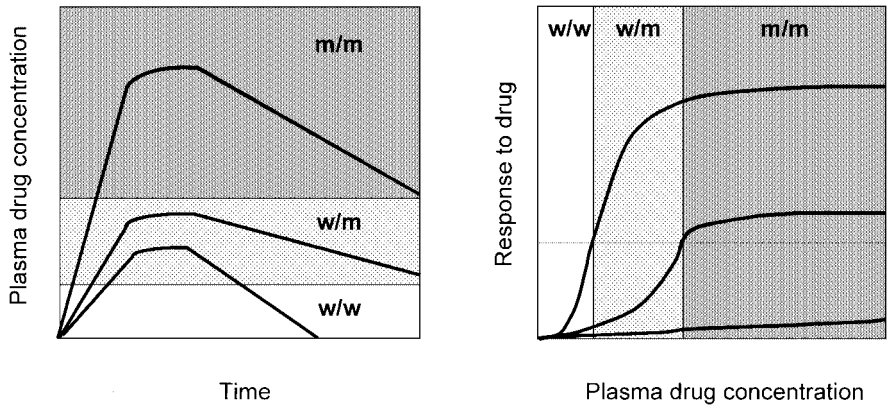

Fig. 7 Relation between drug response and genetic polymorphism.
w, Normal; m, Mutant
w/w, Normal (Extensive metabolizer); m/m, Poor metabolizer
w/m, Heterozygote

Table 2 Genetic polymorphisms in drug metabolizing enzymes

Cytochrome P450 (CYP)				
CYP1A1	CYP1A2	CYP1B1	CYP2A6	CYP2C9
CYP2C18	CYP2C19	CYP2D6	CYP2E1	CYP3A4
Flavin containing monooxygenase (FMO)				
Dihydropyrimidine dehydrogenase (DPD)				
Sulfur transferase (ST)				
Glutathione S-transferase (GST)				
N-acetyltransferase (NAT)				
UDP-glucuronyl transferase (UGT)				
Thiopurine methyltransferase (TPMT)				
Aldehyde dehydrogenase (ALDH)				

more water-soluble compound. Phase III is the elimination process which involves a transporter protein on the cell membrane, and ATP for energy.

The relation between drug response and genetic polymorphism is illustrated as a schematic example in Fig. 7. Most drugs are metabolized in the liver, followed by the kidney, gastrointestinal tracts and in organs where the drug is targeted and functions. It is known that there are extensive metabolizers (EM) who have fast drug metabolism, while a few people are poor metabolizers (PM). This was recognized clearly in the metabolism of isoniazid in the 1960's. Presently, it is clear that the observed difference in isoniazid metabolism, which involves N-acetylation, is due to genetic polymorphism of a CYP gene. Thus, in PM phenotype, there is less CYP activity, resulting in a higher concentration of isoniazid in circulation, and a higher incidence of side-effects, compared with EM phenotype (Fig. 7, left panel). PM may also show side-effects, but not the target effect of the drug (Fig. 7, right panel).

A few examples of genetic polymorphisms in drug metabolizing enzymes are summarized in Table 2. The first three represent Phase I enzymes, while the remaining 6 represent Phase II enzymes. Recently, recognized examples, which influence the drug metabolism, such as pravastatin, albuterol, clozapin and ABT-761, are shown in Table 3. For example, pravastatin, a drug which decreases abnormally increased levels of LDL by inhibiting HMG CoA reductase, is metabolized at various rates depending on genotype of cholesterol ester transfer protein (CETP). At least, three different transporters (liver-specific transporter-1, also referred to as OATP, or LST-1), cMOAT (MRP), and one other which has not been fully characterized. Polymorphism studies at all transporter levels should influence pharmacodynamics, but such information is yet very preliminary, and more transporters should be defined in future studies.

If genetic polymorphisms occur for drug metabolism of target molecules, they may have immediate significant consequences. Some may elicit EM and PM phenotypes, while others may alter pharmacodynamics (Table 4). For example, amino acid substitution at the 64th residue of the β3-adrenergic receptor is known to be associated with insulin-non-dependent juvenile onset diabetes, and insulin resistance (38).

All these phenomena are very similar to the pharmacodynamic activation of the otherwise potentially silent *PBGD* gene defect in AIP, but clinical attacks can be elicited in certain individuals with a susceptible genotype of drug metabolism, while others may fare well against exposure to the same inducing drug. Thus, all these findings suggest that AIP is not strictly a monogenic disorder as has been held for a long time, but there is a significant interplay between the primary gene defect, namely PBGD deficiency, and other factors that facilitate the clinical expression of the PBGD gene defect. These other contributing factors may be

Table 3 Genetic polymorphism and drug response

Drug	*Target molecule*	*Pharmacodynamics/Pharmacogenomics*
Pravastatin	HMG CoA reductase	B1/B1 genotype of CETP: A favorable response to Pravastatin; B2/B2 genotype of CETP: Lack of response
Albuterol	β-Adrenergic receptor	Arg16Gly of βAR decreases sensitivity to Albuterol.
Clozapine	Dopamine D3, D4, 5-$HT_{2A,2C}$ receptors	Ser9Gly of D3: a lack of response to Clozapine. His452Tyr of 5-HT_{2A} receptor: decreased sensitivity.
ABT-761	5-Lipoxygenase	Decreased Sp-1 motifs in the 5-lipoxygenase gene: a decreased response to the drug.

Table 4 Genetic polymorphism of target molecules and their consequences

Drug	*Target molecule*	*Consequence*
Pravastatin	Choresterol ester transfer protein (CETP)	Progression of arteriosclerosis
Albuterol	β-Adrenergic receptor	Decrease in anti-asthma effect
Zileuton	5-Lipoxygenase	Decrease in anti-asthma effect
Tacrine	Choline acetyl transferase	Treatment of Alzheimer's disease/Apo E
Drug with QT prolongation	hERG/MiRP1	Increased risk of *torsades de pointes* in subjects with MiRP1 mutation
Anti-malariacs	Glucose 6-phosphate dehydrogenase	Increased risk of hemolysis
ACE inhibitor/β-blocker	Angiotensin converting enzyme	Decreased response in gene carriers; Increased response in gene carriers
Drug with QT prolongation	KCNQ1/KCNE1	Increased risk of arrhythmia
Fluvastatin	ABCA1 transporter	Decreased response to drug

either acquired or genetic, and their nature can be quite heterogeneous. These findings also predict that, while one may be able to define the existence of the PBGD defect by exact molecular diagnosis, it would be extremely difficult to precisely predict the clinical expression of AIP since it is determined by a combined effect of various genetic or environmental factors. The lessons from AIP are, however, very useful in clarifying the complex nature of the clinical expression of metabolic disorders, and our conclusion should also apply to other genetic disorders.

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References

(1) Garrod AE. The Croonian Lectures on Inborn Errors of Metabolism. Lancet 1908; ii: 1–7.
(2) Sassa S. The Porphyrias. In Williams Hematology, 6th Edition. E. Beutler, M.A. Lichtman, B.S. Coller, T.J. Kipps and U. Seligman, editors. McGraw-Hill, Inc., New York, 2001; 703–720.
(3) Voswinckel P. A constant source of surprises: Acute porphyria. Two cases reported by Hippocrates and Sigmund Freud. History of Psychiiatry 1990; 1: 159–168.
(4) Voswinckel P. Der Schwarze Urin: Vom Schrecknis zum Laborparameter. Blackwell Wissenschafts-Verlag, Berlin, 1992.
(5) Anderson KE, Sassa S, Bishop DF, Desnick RJ. Disorders of heme biosynthesis: X-linked sideroblastic anemia and the porphyrias. In The Metabolic & Molecular Bases of Inherited Disease, 8th Edition. C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, editors. McGraw-Hill Medical Publishing Division, New York, 2001; 2991–3062.
(6) Kondo M, Yano Y, Urata G, Shirataka M. The Porphyrias. In Frontiers of Photobiology. A. Shima, M. Ichihashi, Y. Fujiwara, and H. Takebe, editors. Elsevier Science Publishers, Amsterdam, 1993; 449–454.
(7) Grandchamp B, Beaumont C, de Verneuil H, Walter O, Nordmann Y. Genetic expression of porphobilinogen deaminase and uroporphyrinogen decarboxylase during the erythroid differentiation of mouse erythroleukemic cells. In Porphyrins and Porphyrias. Y. Nordmann, editor. John Libbey & Company Ltd., London, 1986; 35–44.
(8) Romeo PH, Chretien S, Dubart A, Beaupain D, Raich N, Romana M, Grandchamp B, Goossens M. Erythroid specific promoter of an housekeeping gene. Prog. Clin. Biol. Res. 1987; 251: 55–62.
(9) Grandchamp B, Picat C, Mignotte V, Wilson JH, Te VK, Sandkuyl L, Romeo PH, Goossens M, Nordmann Y. Tissue-specific splicing mutation in acute intermittent porphyria. Proc. Natl. Acad. Sci. USA 1989; 86: 661–664.
(10) Grandchamp B, Picat C, de Rooij F, Beaumont C, Wilson P, Deybach JC, Nordmann Y. A point mutation G A in exon 12 of the porphobilinogen deaminase gene results in exon skipping and is responsible for acute intermittent porphyria. Nucl. Acids Res. 1989; 17: 6637–6649.
(11) Anderson KE, Spitz IM, Bardin CW, Kappas A. A gonadotropin releasing hormone analogue prevents cyclical attacks of porphyria. Arch. Int. Med. 1990; 150: 1469–1474.
(12) Anderson KE, Bradlow HL, Sassa S, Kappas A. Studies in porphyria. VIII. Relationship of the 5α-reductive metabolism of steroid hormones to clinical expression of the genetic defect in acute intermittent porphyria. Am. J. Med. 1979; 66: 644–650.
(13) Kauppinen R, Mustajoki P. Prognosis of acute porphyria: occurrence of acute attacks, precipitating factors, and associated diseases. Medicine 1992; 71: 1–13.
(14) Anderson KE, Freddara U, Kappas A. Induction of hepatic cytochrome P-450 by natural steroids: relationship to the induction of δ-aminolevulinate synthase and porphyrin accumulation in the avian embryo. Arch. Biochem. Biophys. 1982; 217: 597–608.
(15) Sassa S, Kappas A. Succinylacetone inhibits δ-aminolevulinate dehydratase and potentiates the drug and steroid induction of δ-aminolevulinate synthase in liver. Trans. Assoc. Am. Physicians 1982; 95: 42–52.
(16) Bottomley SS, Müller-Eberhard U. Pathophysiology of heme synthesis. Semin. Hematol. 1988; 25: 282–302.
(17) Sinclair JF, Sinclair PR, Smith EL, Bement WJ, Pomeroy J, Bonkowsky H. Ethanol-mediated increase in cytochrome P-450 in cultured hepatocytes. Biochem. Pharmacol. 1981; 30: 2805–2809.
(18) White IN, Campbell JB, Farmer PB, Bailey E, Nam NH, Thang DC. Metabolic activation of acetylenes. Covalent binding of [1,2-^{14}C]octyne to protein, DNA and haem in vitro and the protective effects of certain thiol compounds. Biochem. J. 1984; 220: 85–94.
(19) De Matteis F, Marks GS. Cytochrome P450 and its interactions

with the heme biosynthetic pathway. Can. J. Physiol. Pharmacol. 1996; 74: 1–8.
(20) White IN, Muller-Eberhard U. Decreased liver cytochrome P-450 in rats caused by norethindrone or ethynyloestradiol. Biochem. J. 1977; 166: 57–64.
(21) Mustajoki P, Heinonen J. General anesthesia in "inducible" porphyria. Anesthesiology 1980; 53: 15.
(22) Harrison GG, Meissner PN, Hift RJ. Anaesthesia for the porphyric patient. Anaesthesia 1993; 48: 417–421.
(23) Dover SB, Plenderleith L, Moore MR, McColl KE. Safety of general anaesthesia and surgery in acute hepatic porphyria. Gut 1994; 35: 1112–1115.
(24) Samuels B, Bezwoda WR, Derman DP, Goss G. Chemotherapy in porphyria. S. Afr. Med. J. 1984; 65: 924–926.
(25) Gorchein A. Drug treatment in acute porphyria. Br. J. Clin. Pharmac. 1997; 44: 427–434.
(26) Welland FH, Hellman ES, Gaddis EM, Collins A, Hunter GW, Jr, Tschudy DP. Factors affecting the excretion of porphyrin precursors by patients with acute intermittent porphyria. I. The effects of diet. Metabolism 1964; 13: 232–250.
(27) Felsher BF, Redeker AG. Acute intermittent porphyria: Effect of diet and griseofulvin. Medicine 1967; 46: 217–223.
(28) Tschudy DP, Welland FH, Collins A, Hunter G, Jr. The effect of carbohydrate feeding on the induction of δ-aminolevulinic acid synthetase. Metabolism 1964; 13: 396–406.
(29) Anderson KE, Kappas A. Diet regulates drug and hormone metabolism in humans: Experimental findings and clinical implications. In Foreign Compound Metabolism. J. Caldwell and G.D. Paulson, editors. Taylor & Francis, London and Philadelphia. 1984; 299–308.
(30) Pantuck EJ, Pantuck CB, Anderson KE, Conney AH, Kappas A. Cigarette smoking and chlorpromazine disposition and actions. Clin. Pharmacol. Ther. 1982; 31: 533–538.
(31) Lip GY, McColl KE, Goldberg A, Moore MR. Smoking and recurrent attacks of acute intermittent porphyria. Br. Med. J. 1991; 302: 507.
(32) Paxton JR, Moore MR, Beattie AD, Goldberg A. 17-Oxosteroid conjugates in plasma and urine of patients with acute intermittent porphyria. Clin. Sci. Mol. Med. 1974; 46: 207.
(33) Sassa S, Kappas A. Hereditary tyrosinemia and the heme biosynthetic pathway. Profound inhibition of δ-aminolevulinic acid dehydratase activity by succinylacetone. J. Clin. Invest. 1983; 71: 625–634.
(34) Sassa S, Fujita H, Kappas A. Succinylacetone and δ-aminolevulinic acid dehydratase in hereditary tyrosinemia: immunochemical study of the enzyme. Pediatrics 1990; 86: 84–86.
(35) Lindblad B, Lindstedt S, Steen G. On the genetic defects in hereditary tyrosinemia. Proc. Natl. Acad. Sci. USA 1977; 74: 4641–4645.
(36) Gorchein A. Drug treatment of hypertension in acute intermittent porphyria: doxazosin and amlodipine. Br. J. Clin. Pharmac. 1997; 43: 339–340.
(37) Kepple A, Cernek PK. Amlodipine-induced acute intermittent porphyria exacerbation. Annal. Pharmacother. 1997; 31: 253.
(38) Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC. Association of a polymorphism in the beta 3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N. Engl. J. Med. 1995; 333: 348–351.
(39) Kappas A, Sassa S, Galbraith RA, Nordmann Y. The porphyrias. In The Metabolic and Molecular Basis of Inherited Disease, 7th Edition. C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, editors. McGraw-Hill, Inc., New York. 1995; 2103–2159.